



PATENT COOPERATION TREATY
PCT
INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference P6000290PCT	FOR FURTHER ACTION		See Form PCT/PEA/416
International application No. PCT/EP2004/002987	International filing date (<i>day/month/year</i>) 19.03.2004	Priority date (<i>day/month/year</i>) 19.03.2004	
International Patent Classification (IPC) or national classification and IPC INV. A23L1/305 A23L1/30 A23C9/123 A23C9/127 A23L1/211 A61K38/01			
Applicant CAMPINA NEDERLAND HOLDING B.V.et al			
<p>1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 6 sheets, including this cover sheet.</p> <p>3. This report is also accompanied by ANNEXES, comprising:</p> <p style="margin-left: 20px;">a. <input checked="" type="checkbox"/> <i>sent to the applicant and to the International Bureau</i> a total of 2 sheets, as follows:</p> <p style="margin-left: 40px;"><input checked="" type="checkbox"/> sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).</p> <p style="margin-left: 40px;"><input type="checkbox"/> sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.</p> <p style="margin-left: 20px;">b. <input type="checkbox"/> (<i>sent to the International Bureau only</i>) a total of (indicate type and number of electronic carrier(s)) , containing sequence listing and/or tables related thereto, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).</p>			
<p>4. This report contains indications relating to the following items:</p> <p><input checked="" type="checkbox"/> Box No. I Basis of the report</p> <p><input type="checkbox"/> Box No. II Priority</p> <p><input type="checkbox"/> Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</p> <p><input type="checkbox"/> Box No. IV Lack of unity of invention</p> <p><input checked="" type="checkbox"/> Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</p> <p><input type="checkbox"/> Box No. VI Certain documents cited</p> <p><input type="checkbox"/> Box No. VII Certain defects in the international application</p> <p><input type="checkbox"/> Box No. VIII Certain observations on the international application</p>			
Date of submission of the demand 13.07.2005		Date of completion of this report 04.07.2006	
Name and mailing address of the international preliminary examining authority:  European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016		Authorized officer De Jong, E Telephone No. +31 70 340-3849 	

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INTERNATIONAL PRELIMINARY REPORT
ON PATENTABILITYInternational application No.
PCT/EP2004/002987

Box No. | Basis of the report

1. With regard to the language, this report is based on

- ☒ the international application in the language in which it was filed
- ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of:
- ☐ international search (under Rules 12.3(a) and 23.1(b))
 - ☐ publication of the international application (under Rule 12.4(a))
 - ☐ international preliminary examination (under Rules 55.2(a) and/or 55.3(a))

2. With regard to the elements* of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report):***Description, Pages**

1-11 as originally filed

Claims, Numbers

1-9 received on 13.07.2005 with letter of 13.07.2005

Drawings, Sheets

1/5-5/5 as originally filed

- ☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:

- ☐ the description, pages
- ☐ the claims, Nos.
- ☐ the drawings, sheets/figs
- ☐ the sequence listing (*specify*):
- ☐ any table(s) related to sequence listing (*specify*):

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

- ☐ the description, pages
- ☐ the claims, Nos.
- ☐ the drawings, sheets/figs
- ☐ the sequence listing (*specify*):
- ☐ any table(s) related to sequence listing (*specify*):

* If item 4 applies, some or all of these sheets may be marked "superseded."

**INTERNATIONAL PRELIMINARY REPORT
ON PATENTABILITY**

International application No.
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Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes: Claims	1-6,9
	No: Claims	7,8
Inventive step (IS)	Yes: Claims	1-6
	No: Claims	7-9
Industrial applicability (IA)	Yes: Claims	1-9
	No: Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

**INTERNATIONAL PRELIMINARY
REPORT ON PATENTABILITY
(SEPARATE SHEET)**

International application No.

PCT/EP2004/002987

Ad V**1. Reference is made to the following documents:**

- D3: WO 02/071854 A (UNILEVER PLC ; LEVER HINDUSTAN LTD (IN); UNILEVER NV (NL)) 19 September 2002 (2002-09-19)
- D4: EP-A-1 018 341 (CALPIS CO LTD) 12 July 2000 (2000-07-12)
- D6: GOBBETTI M ET AL: "Production of angiotensin-I-converting-enzyme-inhibitory peptides in fermented milks started by *Lactobacillus delbrueckii* subsp. *bulgaricus* SS1 and *Lactococcus lactis* subsp. *cremoris* FT4." APPLIED AND ENVIRONMENTAL MICROBIOLOGY 66 (9) 3898-3904 2000 DIPARTIMENTO DI SCI. DEGLI ALIMENTI, SEZIONE DI MICROBIOL. AGRO-ALIMENTARE, UNIV. DEGLI STUDI DI PERUGIA, S. CONSTANZO, 06126 PERUGIA, ITALY. TEL./FAX 39 75 32387. E-MAIL GOBBETTI(A)UNIPG, 2000, XP002306642
- D7: FUGLSANG A ET AL: "Cardiovascular effects of fermented milk containing angiotensin-converting enzyme inhibitors evaluated in permanently catheterized, spontaneously hypertensive rats" APPLIED AND ENVIRONMENTAL MICROBIOLOGY, WASHINGTON,DC, US, vol. 68, no. 7, 1 July 2002 (2002-07-01), pages 3566-3569, XP002252114 ISSN: 0099-2240
- D8: US-B-6 514 9411 (VAN DER VEERDONK FRANS ET AL) 4 February 2003 (2003-02-04)
- D9: TAUZIN J ET AL: "Angiotensin-I-converting enzyme inhibitory peptides from tryptic hydrolysate of bovine alphaS2-casein" FEBS LETTERS, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 531, no. 2, 6 November 2002 (2002-11-06), pages 369-374, XP004391663 ISSN: 0014-5793
- D10: FAN J ET AL: "PREPARATION OF ANGIOTENSIN I-CONVERTING ENZYME INHIBITING PEPTIDES FROM SOYBEAN PROTEIN BY ENZYMATIC HYDROLYSIS" FOOD SCIENCE AND TECHNOLOGY RESEARCH, XX, XX, vol. 9, no. 3, 2003, pages 254-256, XP009029152
- D11: AHN W-W ET AL: "Isolation of Angiotensin I Converting Enzyme Inhibitory Peptide from Soybean Hydrolysate" FOOD SCIENCE AND BIOTECHNOLOGY, KOREAN SOCIETY OF FOOD SCIENCE AND TECHNOLOGY, SEOUL, KR, vol. 9, no. 6, 2000, pages 378-381, XP009029145 ISSN: 1226-7708

**INTERNATIONAL PRELIMINARY
REPORT ON PATENTABILITY
(SEPARATE SHEET)**

International application No.

PCT/EP2004/002987

D13: EP-A-0 406 598 (NESTLE SA) 9 January 1991 (1991-01-09)
D14: NL-A-9 301 525 (SNOW BRAND EUROP RESEARCH LAB) 3 April 1995
(1995-04-03)

2. The amendments filed with the letter 13.07.2005 are considered to comply with Article 34(2)(b) PCT.

3. The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims 7 and 8 is not new in the sense of Article 33(2) PCT and claim 9 not inventive in the sense of Article 33(3) PCT:

D3 discloses (see claims 1-19) a fermented milk product (e.g. drink, yoghurt, cheese, spread) having an ACE inhibitory effect, produced from milk fermented with *Lactobacillus delbrueckii* subsp. *lactis*. The food product contains peptides having 2-15, or 6-10 amino acids. The proteins are hydrolysed by fermentation.

D3 is considered to anticipate the subject-matter of claims 7 and 8.

D4 discloses (see p.3 par.18-22) fermentation of a food material (e.g. milk, soybean, corn) by *Lactobacilli* (e.g. *Lb. acidophilus*, *Lb. casei*), to obtain tripeptide having angiotensin converting enzyme inhibitory activity. The functional food of the invention of D4 may include e.g. yogurt (par.34). The proteins are hydrolysed by fermentation.

D4 is considered to anticipate the subject-matter of claims 7 and 8.

D6 discloses (p.3899 col.1 par.5) fermentation of UHT skim milk by *L. delbrueckii* subsp. *bulgaricus* and *L. lactis* subsp. *cremoris* FT4, to produce fermented milk containing ACE inhibitory activity (p.3900 col.1). The proteins are hydrolysed by fermentation.

D6 is considered to anticipate the subject-matter of claim 7.

The same applies to D7, using *L. helveticus*.

The subject-matter of claim 9 is not considered to involve an inventive step in view of the same documents, because the modification to for example kefir comes within the scope of the customary practice followed by persons skilled in the art.

4. The subject-matter of claims 1-6 is considered to be both novel and to involve an inventive step:

The document D8 is regarded as being the closest prior art and discloses (see claims 1-8 and Examples 1-6) a casein hydrolysate, prepared by tryptic hydrolysis, that is enriched in antihypertensive peptides called C6, C7 and C12.

D9 refers to the same process and D10/D11 to enzymatic (Protease M, Orientase 90N and protease derived from *B. subtilis*) hydrolysis of soybean protein to obtain ACE inhibiting peptides.

In the processes of D8-D11 no fermentation of the hydrolysates takes place.

The problem to be solved by the present invention may therefore be regarded as an optimisation of the process/product in terms of taste.

D13 and D14 both teach the use of lactobacilli for debittering; *Lb. helveticus* is stated to have the best effect, but other lactobacilli are mentioned too (see Fig.3 of D14).

However, the person skilled in the art would not have used the teachings of D13/D14, because he would expect loss of biofunctionality when ACE-inhibiting peptides are hydrolysed.

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CLAIMS

1. Method of preparing a food product or food ingredient having angiotensin-I-converting enzyme inhibiting properties, which method comprises:
 - a) providing a preparation of one or more protein hydrolysates having angiotensin-I-converting enzyme inhibiting properties
 - b) mixing the preparation thus provided with a constituent selected from whole milk, low-fat milk, non-fat milk, cream and recombined milk;
 - c) adding one or more microorganism species to the mixture thus obtained and
 - d) fermenting the mixture.
2. Method as claimed in claim 1, wherein the protein hydrolysate comprises casein hydrolysate.
3. Method as claimed in claim 2, wherein the casein hydrolysate is a hydrolysate containing C6, C7 and/or C12 peptides.
4. (8) Method of preparing a food product having angiotensin-I-converting enzyme inhibiting properties, which method comprises:
 - a) providing a starting material for the food product selected from whole milk, low-fat milk, non-fat milk, cream and recombined milk;
 - b) hydrolysing proteins contained in the starting material to produce a hydrolysate having angiotensin-I-converting enzyme inhibiting properties;
 - c) adding one or more fermenting microorganisms to the mixture thus obtained; and
 - d) fermenting the mixture.

5. Method as claimed in any one of the claims 1-4, wherein the microorganisms are selected from the group consisting of *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, *Lactobacillus acidophilus*, *Lactobacillus casei* and *Bifidobacterium bifidum*.
6. Method as claimed in any one of the claims 1-4, wherein fermenting is continued for a period that is longer than the time normally required for optimal growth of the fermenting microorganism.
7. Food product or food ingredient having angiotensin-I-converting enzyme inhibiting properties, obtainable by the method according to any one of claims 1-4.
8. Food product as claimed in claim 7, which is a yoghurt.
9. Food product as claimed in claim 7, which is selected from kefir, acidophilus milk, cultured cream and koumiss.

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